

Exhibit L

Shrinking of Polypropylene Mesh in vivo: An Experimental Study in Dogs*

U. Klinge,¹ B. Klosterhalfen,² M. Müller,¹ A. P. Öttinger³ and V. Schumpelick¹

From the ¹Department of Surgery of the RWTH Aachen, ²Institute for Pathology of the RWTH Aachen, Aachen, Germany and ³Joint Institute for Surgical Research, Moscow, Russia

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ABSTRACT

Objective: To assess the extent of shrinkage of meshes used for hernia repair.

Design: Experimental study in dogs.

Setting: University hospital, Germany and University Research Centre, Moscow.

Animals: 10 dogs had monofilament polypropylene meshes that weighed 95 g/m² (Marlex[®]) or multifilament reduced polypropylene meshes combined with polyglactin 910 that weighed 55 g/m² (Soft Hernia Mesh[®]) implanted for either 3 or 6 months.

Main outcome measures: Histological appearance and radiological assessment of the position and area of the mesh.

Results: After 4 weeks the area of mesh in the monofilament group was reduced from 139 (11) to 75 (8) cm² (54%) and that of the multifilament from 116 (18) to 77 (20) cm² (66%). The multifilament mesh with the reduced amount of polypropylene showed less inflammatory response and less shrinkage. The mesh did not seem to have moved.

Conclusion: Meshes that contain a lot of polypropylene shrink to about 30%–50% of their original size after 4 weeks, requiring an overlap of at least 3 cm if implanted subfascially. Reduction in the polypropylene content decreases both the inflammatory response and the shrinkage. Meshes with big pores are less likely to fold and improve compatibility.

Key words: mesh shrinking, polypropylene, wound contraction, hernia repair.

INTRODUCTION

In hernia surgery meshes are essential to achieve a strong repair of the abdominal wall. The resulting scar decreases the recurrence rate but is often accompanied by appreciable complaints. (2, 3, 10). The appearance of dislocated mesh in bladder and bowel (5, 6, 13) as well as the histological examination of removed meshes have shown that the incorporated alloplastic material is not inert and causes a constant inflammatory response, folding and shrinking. To evaluate the compatibility of nonabsorbable meshes we implanted both monofilament and multifilament polypropylene meshes in dogs. The position and extent of the meshes were followed radiographically.

MATERIAL AND METHODS

We implanted two different meshes in dogs (weighing 15 (2) kg) for 3 (n = 6) and 6 months (n = 4). The experiments followed the NIH guidelines for animal

studies. In one group we used a monofilament polypropylene mesh (Marlex[®], Bard), and in the second a multifilament combination of a reduced amount of nonabsorbable polypropylene (27%) and absorbable polyglactin 910 (Soft Hernia Mesh[®], Ethicon) (Table I).

The meshes measured 8 × 10 cm and were implanted preperitoneally on the posterior sheet of the rectus fascia and fixed in place with single sutures of 2/0 polypropylene. Both the mesh and the adjacent soft tissue were marked with pairs of metallic clips, three pairs on each side. The fascia was closed with a running suture of 2/0 polypropylene and the skin with a continuous 3/0 silk suture.

We followed the position of the mesh and calculated its size on anteroposterior radiographs taken on day 0, 7, 14, 28, 56, 74 and 148. The radiographs were taken with the animals supine and sedated with 10% ketamine 3–4 ml. The distance between the animal and the x-ray machine was 45 cm (magnifying factor 1.5). We measured the length of the four sides and of the two oblique lines. The area of mesh was calculated as a composition of one rectangle and three triangles.

Finally the animals were killed and the complete abdominal wall removed. The four edges of the mesh were cut for histological examination. The remaining

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Table I. Textile analysis of polypropylene meshes (n = 10 in each group)

| | Monofilament | Multifilament |
|--|--------------|---------------|
| Weight (g/m ²) | 95 | 55 |
| Thickness (mm) | 0.7 | 0.7 |
| Percentage of pores | 85 | 91 |
| Bending stiffness (N/cm ²) | 0.35 | 0.07 |
| Tear out test of seam (N) | | |
| Vertical | 57 | 30 |
| Horizontal | 56 | 29 |
| Subsequent tear-out strength (N) | | |
| Vertical | 7 | 11 |
| Horizontal | 40 | 12 |
| Maximum pulling force (N/5cm) | | |
| Vertical | 432 | 387 |
| Horizontal | 567 | 627 |
| Testing the pressing through the stamp | | |
| Tensile strength (N/cm) | 59 | 32 |

part was stored in saline and tested mechanically within 24 hours.

Conventional light microscopy was done on 4 µm slices stained with haematoxylin and eosin, Giemsa, and van Gieson after they had been fixed in formalin and embedded in paraffin. Morphometric analysis was made at the interface within 500 µm around the mesh fibres. Finally, we calculated the partial volume and the percentage of cells. To evaluate the shrinking of the meshes we compared the width of the pores over a distance of 2 cm in 10 slices/animal.

Statistical analysis was done using the software Statistical package for the Social Sciences (SPSS 5.0.1[®] for windows).

RESULTS

Local infections developed in 3 animals in the monofilament group and one in the multifilament group. One animal with a monofilament mesh died of septic complications. In the multifilament group there was one haematoma, and one asymptomatic abscess after six months.

After mesh had been removed we saw obvious folding of the material, crosswise to the filaments in three of five animals with the monofilament polypropylene material, which resulted in a pronounced increase in bending stiffness (Fig. 1).

The radiographic examination of pairs of clips (259/360 were detected) in situ showed neither a significant increase in the distance between tissue and mesh clips nor a significant difference between both materials and time after implantation (simple factorial ANOVA). The biggest differences were recorded 4–8 weeks after implantation.

The two-dimensional analysis of the mesh area in the monofilament group showed shortening by 25% in both horizontal and vertical directions, as well as a shrinking of the mesh area of 46% after four weeks (Table II). The shrinking of the mesh with the reduced amount of polypropylene (multifilament) was less, having decreased by 12% in the horizontal and 25% in the vertical plane, which means a reduction in the area of 34% (Fig. 2).

The textile analysis of the whole removed abdominal wall by pressing it through a stamp (small stamp, velocity 50 mm/min, clamping length 40 mm, tested area 9.35 cm²) was possible in one only animal of each

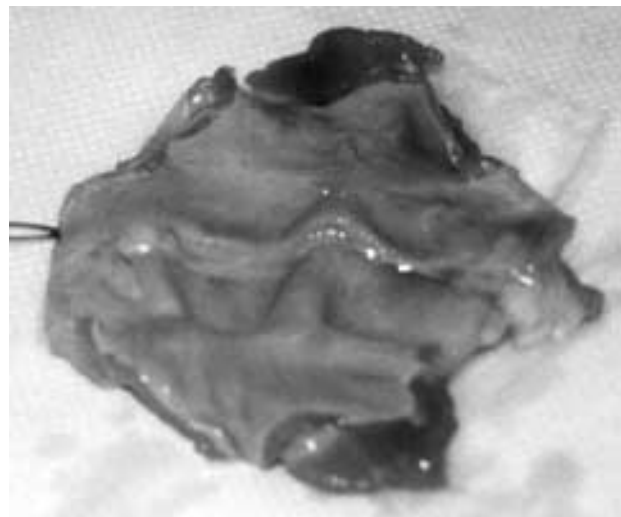


Fig. 1. Removed monofilament polypropylene mesh after 3 months.

Table II. Mean (SD) area (cm²) of meshes implanted in dogs measured on radiographs

| Day | Monofilament | | Multifilament | |
|-----|--------------|----------|---------------|----------|
| | No. | Area | No. | Area |
| 0 | 5 | 139 (11) | 5 | 116 (18) |
| 7 | 5 | 119 (13) | 5 | 110 (16) |
| 14 | 4 | 71 (16) | 5 | 85 (9) |
| 28 | 4 | 75 (8) | 5 | 78 (20) |
| 56 | 4 | 45 (55) | 5 | 74 (23) |
| 84 | 1 | 81 | 2 | 57/98 |
| 168 | 1 | 63 | 2 | 70/97 |

group because of the extensive shrinking of the mesh. Despite the differences in weight the rupture strength was similar: for the monofilament mesh 1950 N when stretched by 15 mm and for the multifilament mesh 2040 N at a stretching of 13 mm.

HISTOLOGICAL EXAMINATION

Monofilament polypropylene mesh

After three months we saw a predominant acute and productive inflammation with typical foreign body granulomas including epithelioid cells and giant cells. Adjacent to the mesh fibres there were parallel orientated areas of fibrinoid necrosis with a dense infiltration of polymorphonuclear leucocytes. Lymphocytes and plasma cells were seen mainly in the outlying area of the granulomas. There was pronounced perifilamentous fibrosis with extensive collagen fibres which were mainly orientated parallel to the polypropylene fibres adjacent to the mesh. Further away they formed an all embedding capsule. After three months fibroblasts were still common, whereas vascular structures were rare. After six months the inflammatory reaction was reduced but still visible within the

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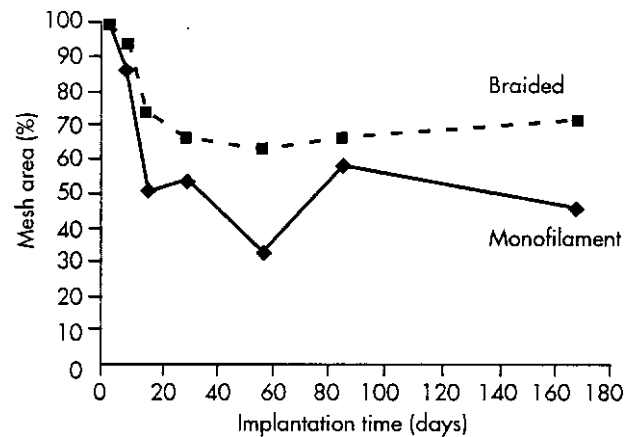


Fig. 2. Percentage mesh area plotted over time (radiograph, dogs).

granulomas, which showed signs of maturation with a slightly increased number of giant cells. The fibrosis seemed to be constant, and the necrosis had totally vanished.

Multifilament mesh with reduced amount of polypropylene combined with polyglactin 910

The inflammatory response was considerably less than in the monofilament group. The reaction was characterised by granulomas and polymorphonuclear giant cells. Acute inflammation was rare with hardly any fibrinoid necrosis. Some residual fragments of the absorbable polyglactin 910 were still visible. In general the fibrosis was moderate. The collagen fibres were orientated around the mesh filaments. There was no scar plate as seen in the monofilament group. There were few fibroblasts and increased vascularisation. After six months the histological appearance remained unchanged with minimal inflammatory signs, a slight decrease in inflammatory cells, and a minimal increase in fibrosis and giant cells without necrosis.

Table III. Morphometric assessment of meshes implanted for three and six months. Data are expressed as mean (SD) except where otherwise stated

| Mesh material | Three months | | Six months | |
|---------------------------------------|----------------------|-----------------------|----------------------|-----------------------|
| | Monofilament (n = 6) | Multifilament (n = 6) | Monofilament (n = 6) | Multifilament (n = 6) |
| Inflammatory cells (% partial volume) | 24 (2) | 14 (4) | 21 (3) | 8 (1) |
| Soft tissue cells (% partial volume) | 57 (15) | 29 (7) | 61 (10) | 30 (5) |
| Fat cells (% partial volume) | 32 (6) | 78 (20) | 28 (8) | 79 (22) |
| Vascular cells (% partial volume) | 17 (4) | 37 (8) | 14 (2) | 33 (12) |
| Polymorph leucocytes % | 11 (2) | 4 (1) | 6 (3) | 1 (1) |
| Giant cells % | 3 (1) | 10 (2) | 5 (1) | 13 (1) |
| Macrophages % | 36 (12) | 45 (13) | 38 (6) | 42 (8) |
| Pore size mm | 2 | 5 | 2 | 4 |
| (% of initial material) | 81 | 93 | 73 | 83 |

In general the main tissue reaction in both groups seemed to be completed after three months (Table III).

After 6 months the pores width of the multifilament mesh had markedly decreased from initially 2.54 ± 0.46 mm to 1.85 ± 0.28 mm ($p < 0.01$). This corresponded to a reduced length of 27% and a reduced area of 47%. The pore size of the mesh with reduced amount of polypropylene decreased from 4.99 ± 0.56 mm to 4.15 ± 0.41 mm ($p < 0.01$) corresponding to a shortening of 16% and a shrinking of the mesh area of 28%. This correlates directly to the data of the X-ray analysis.

DISCUSSION

Mesh material implanted in dogs for three to six months shrank considerably over time. Although a two-dimensional calculation of the area of the mesh neglects the curvature and underestimates the size, significant shrinkage could be shown on both radiographic and histological examination compared with the initial size on day 0.

The quantity and structure of alloplastic material dictates the extent of the inflammatory response, the induction of scar tissue, and therefore the mechanical function of the abdominal wall. Experiments in rats have confirmed the correlation between weight of the mesh and bending stiffness of the abdominal wall (7). The reduction in the amount of nonabsorbable polypropylene reduces the extent of the inflammatory response and the corresponding consecutive fibrosis (9). Our histological examination in dogs confirmed the reduction in inflammation when a mesh with a reduced amount of polypropylene was used.

The present data confirm the results of Amid, who described a shortening of the mesh of 20% within 10 months (1). This corresponds with a reduced area of about 64%. Shrinking might be responsible for secondary folding in cases of poor elasticity and small pores. It is a consequence of the physiological wound contraction (11), initially by dehydration of soft tissue and later by maturation and crosslinking of the collagen fibres (4). The contraction depends on the extent of inflammation and scar formation resulting from the material used. We have recently published a case report of mesh shrinkage with a resulting secondary hernia and chronic inguinal pain after transabdominal preperitoneal placement of prosthesis (12). Textile analysis showed that many meshes introduced clinically are too strong for the physiological forces of about 16 N/cm at the abdominal wall (8). Improved compatibility can therefore be achieved by using meshes with less alloplastic material, optimised structure (more elasticity and larger pores) and at the same time having sufficient tensile strength.

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RÉSUMÉ

But: Déterminer le degré de rétrécissement des prothèses utilisées pour les cures de hernie.

Type d'étude: Expérimentale chez le chien.

Provenance: Hôpital universitaire, Allemagne et centre de recherche universitaire, Moscou.

Animaux: Dix chiens chez lesquels a été mis en place pour 3 à 6 mois une prothèse en monofilament de polypropylène de 95 g/m² (Marlex®) ou une prothèse composite de 55 g/m² associant polypropylène et polyglactine 910.

Principaux critères de jugement: Les aspects histologiques et la position et la surface de la prothèse déterminées radiologiquement.

Résultats: Après 4 semaines, la surface des prothèses du groupe monofilament avait diminué de 139 (11) à 75 (8) cm².

(54%) et celle du groupe des prothèses composites de 116 (18) à 77 (20) cm² (66%). Les prothèses composites avec une quantité plus faible de polypropylène provoquaient une réponse inflammatoire moindre et rétrécissaient moins. Les prothèses ne semblaient pas s'être déplacées.

Conclusions: Les prothèses qui contiennent une certaine quantité de polypropylène se rétractent d'environ 30 à 50% de leur taille initiale après 4 semaines, ce qui impose de les faire dépasser de 3 cm si elle sont disposées en sous péritonéal. Une proportion moindre de polypropylène diminue à la fois la réponse inflammatoire et le degré de rétrécissement. Les prothèses à larges mailles sont moins susceptibles de se replier sur elles mêmes et s'intègrent mieux.

Mots-clés: Rétrécissement prothétique, polypropylène, rétraction cicatricielle, cure de hernie.

ZUSAMMENFASSUNG

Ziel: Untersuchung des Ausmaßes des Schrumpfens von Netzen in der Hernienchirurgie.

Studienanordnung: Experimentelle Studie an Hunden.

Studienort: Universitätsklinik Deutschland und Universitäts Forschungszentrum, Moskau.

Tiere: 10 Hunde erhielten entweder ein monofiles Polypropylenetz mit einem Gewicht von 95 g/m² (Marlex[®]) oder ein multifiles reduziertes Polypropylenetz kombiniert mit Polyglactin 910 mit einem Gewicht von 55 g/m² für jeweils 3 oder 6 Monate.

Endpunkte: Histologisches Erscheinungsbild und radiologische Untersuchung der Netzposition und des Netzes selbst.

Ergebnisse: Nach 4 Wochen hatte sich die Netzgröße in der monofilen Gruppe von 139 (11) auf 75 (8) cm² (54%) verringert. In der multifilen Gruppe hatte sich die Netzgröße von 116 (18) auf 77 (20) cm² (66%) verringert. Die multifilen Netze mit dem reduzierten Anteil von Polypropylen zeigten weniger entzündliche Antwort und eine geringere Schrumpfung. Die Position der Netze schien sich nicht verändert zu haben.

Schlußfolgerung: Netze mit einem hohen Anteil von Polypropylen schrumpfen auf etwa 30–50% ihrer Originalgröße innerhalb von 4 Wochen, sodaß ein Überlappen von etwa 3 cm bei subfascialer Implantation erforderlich ist. Eine Reduktion des Polypropylenanteils vermindert sowohl die entzündliche Antwort als auch das Schrumpfen. Großporige Netze neigen weniger zum Fallen und zeigen eine verbesserte Kompatibilität.

Schlüsselwörter: Netzschrumpfung, Polypropylen, Wundretraktion, Hernienchirurgie.

РЕЗЮМЕ

Цель: Изучить процент “сморщивания” искусственной сети, используемой для лечения грыжи.

Характер исследования: Экспериментальное исследование на животных (собаки).

Клиника: Университетский госпиталь Германии и Университетский научный центр в Москва.

Животные: 10 собак, у которых была произведена пластика полипропиленовой сетью с весом 95 г/м² (Бфкдуч) или же мультифиламентной редуцированной полипропиленовой сетью, состоящей из комбинаций полиглактина 910 со средним весом 54.55 г/м². Сети были имплантированы на 3 или на 6 месяцев.

Задачи исследования: Гистологическое исследование, а также рентгенологическое исследование для определения позиций сетки.

Результаты: После 4 недель отмечена редукция монофиламентной сети из группы А от 139 до 75 см² (54%), а в группе мультифиламентной сети от 116 до 77 см² (66%). Мультифиламентная сеть с уменьшенным количеством полипропилена показывала меньшую воспалительную реакцию и меньшее “сморщивание”. Не было отмечено случаев дислокации сети.

Выводы: Искусственная сеть, состоящая из полипропилена “сморщивается” приблизительно на 30-40% своего первоначального размера после 4 недель. Уменьшение содержания полипропиленовых волокон позволяет уменьшить процент инфламаторного ответа и “сморщивания”. Сеть с крупными порами лучше переносима организмом.

Ключевые слова: “сморщивание” сети, полипропилен, закрытие раны, герниопластика.

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Address for correspondence:

U. Klinge, M.D.

Department of Surgery of the Technical University Aachen

Pauwelsstr. 30

DE-52074 Aachen

Germany